

## LETTER TO JMG

# Polymorphisms in the mannose binding lectin gene affect the cystic fibrosis pulmonary phenotype

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Cystic fibrosis is a common lethal autosomal recessive disease affecting whites with an incidence of about 1 in 2500. The median lifespan is approximately 30 years. Chronic obstruction and infection of the respiratory tract, pancreatic insufficiency, elevated sweat electrolyte concentration, and male infertility characterise this disease. Clinical manifestations are attributed to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene,<sup>1</sup> which encodes an epithelial chloride channel.<sup>2</sup> Certain phenotypes of cystic fibrosis, such as pancreatic insufficiency, are highly associated with the CFTR genotype. Pulmonary symptoms are highly variable, even among patients from the same family.<sup>3</sup> Other genetic factors, as well as environmental factors, thus affect the cystic fibrosis disease phenotype.

In association studies, evidence has been found that mannose binding lectin (MBL) protein affects cystic fibrosis disease.<sup>4–5</sup> MBL protein is an important mediator component of the innate immune defense system, which functions as an opsonin and complement activator. It is part of a family of proteins called collectins, because they contain collagen-like regions and lectin domains. Through the lectin domain, they bind to carbohydrate structures presented by a wide range of pathogenic bacteria, viruses, fungi, and parasites. Mannose binding lectin protein is synthesised in the liver by hepatocytes and secreted in the blood, and circulates as dimers or hexamers composed of subunits containing three identical polypeptides.<sup>6–7</sup>

The MBL2 gene is located on chromosome 10q11.2–q21. Three single polymorphic MBL2 alleles are known, which are either caused by a nucleotide substitution in codon 52 (arginine with cysteine, allele *D*), codon 54 (glycine with aspartic acid, allele *B*) or codon 57 (glycine with glutamic acid, allele *C*).<sup>8–10</sup> These amino acid changes are thought to affect the tertiary structure of the MBL protein collagenous region. Heterozygosity or homozygosity for these mutations results in little or no functional MBL protein, and hence these alleles have been named *O* (null) alleles.<sup>8–11</sup> Therefore, the common designation for the presence of any of the mutant null alleles is *O* whereas the normal allele has been named *A*.<sup>4–8</sup> Several polymorphisms in the promoter region of MBL2 gene also affect MBL protein serum levels. In particular, the *Y/X* promoter polymorphism affects serum levels of the MBL protein. The *Y* allele is associated with higher plasma levels of MBL protein; the *X* allele is associated with lower MBL protein plasma levels.<sup>4–11</sup>

MBL2 low producer genotypes (when homozygous or heterozygous for the MBL2 *O* allele) have been associated with poor prognosis and early death in patients with cystic fibrosis.<sup>4–5</sup> However, a modulating role of a genetic factor with disease, based on association studies, should only be treated as tentative until the finding of an association has been replicated in other studies. We therefore investigated whether MBL2 structural variants are associated with pulmonary function or susceptibility to *Pseudomonas aeruginosa*

## Key points

- Mannose binding lectin (MBL2) has been proposed to modulate disease severity in cystic fibrosis; however association studies should be treated as tentative until such an association has been replicated in other studies. We therefore investigated whether MBL2 gene variants are associated with pulmonary function or susceptibility to *Pseudomonas aeruginosa* infection in Belgian and Czech patients with cystic fibrosis.
- MBL2 promoter and structural variants were typed by single base primer extension assays in 112 patients with cystic fibrosis and 187 healthy controls. Spirometric data and first age of infection with *Pseudomonas aeruginosa* were collected retrospectively from patients' medical records, to see if they are associated with cystic fibrosis lung disease severity.
- An association was found for the MBL2 structural variants and severity of cystic fibrosis lung disease. Patients having the MBL2 AO or OO genotypes were more likely to have a more severe pulmonary phenotype than patients having the AA genotype ( $p=0.002$ ). No association was found between the MBL2 genotype and the first age of infection with *P. aeruginosa*.
- This study confirms an association between MBL2 variants and the cystic fibrosis pulmonary phenotype, and therefore it is very likely that mannose binding lectin protein is indeed a modulating factor in cystic fibrosis disease.

infection in different ethnic groups of cystic fibrosis patients.

## METHODS

### Patients and control subjects

Only cystic fibrosis patients homozygous for the F508del CFTR mutation were included in the study. We recruited 57 patients with cystic fibrosis from the Belgium University Hospital Gasthuisberg and 122 patients with cystic fibrosis from the Czech Republic Cystic Fibrosis Centre at Prague University Hospital Motol. The control population comprised 85 healthy Belgian adult blood donors, 54 healthy Czech adult blood donors and 48 newborns from the Czech Republic. Controls were ethnically matched to patients. For

**Abbreviations:** CFTR, cystic fibrosis transmembrane conductance regulator; MBL, MBL2, mannose binding lectin; SNP, single nucleotide polymorphism

**Table 1** Primers for PCR amplification of MBL2 fragments

Primer*	Accession number	Nucleotide sequence (5'→3')	Primer concentration	Annealing temperature	PCR product length
47.5	AF080508	CAACGTAGTAAGAAATTCAGAG	0.4 µM	57°C	913 bp
959.3	AF080508	CCAGGCAGTTCTCTGGAAGG	0.4 µM		
621.5	AF080508	ACCCAGATTGTAGGACAGAG	0.1 µM	55°C	339 bp
959.3	AF080508	CCAGGCAGTTCTCTGGAAGG	0.1 µM		

\*The number preceding the decimal point refers to the nucleotide position of the Genbank DNA sequence of the specified accession number given in the neighbouring column, which also corresponds to the 5' nucleotide of the oligonucleotide. The annotations .5 and .3 refer to forward and reverse primers, respectively.

the association study of MBL2 with the disease phenotype, only patients between the ages of 12 and 15 years (mean age 13.4 years) were included (40 Belgian and 72 Czech patients); 48% were male and 52% were female. All clinical data on patients were retrieved from patients' case records. FEV<sub>1</sub>% predicted values were calculated according to Knudson et al.<sup>12</sup> Statistical analysis of lung function was performed on the last FEV<sub>1</sub>% predicted value between the ages of 12 and 15 years. Ages of first infection with *P. aeruginosa* were obtained from the total patient group, which were available from the case records of 116 patients with cystic fibrosis. Patients are followed at the outpatient clinic three monthly. At every visit a sputum sample (or throat swab in patients who do not expectorate) is taken. The age at which *P. aeruginosa* is isolated for the first time is referred to as first infection with *P. aeruginosa*. Association studies involving human subjects have been approved by the ethical committees of both universities.

#### PCR and single nucleotide extension assay

We developed multiplex PCR and multiplex single nucleotide primer extension assays, in which single nucleotide polymorphisms (SNPs) of MBL2 were included. The primers used for amplification of the genomic regions covering the SNPs in MBL2, and the single nucleotide primer extension oligonucleotides for the SNPs in these regions are given in tables 1 and 2, respectively.

PCR reactions were performed in a volume of 50 µl containing 1×Gold Buffer (Applied Biosystems), 0.2 mM dNTPs, 2 mM MgCl<sub>2</sub>, 2.5 U AmpliTaq Gold (Applied Biosystems) and 0.5–1.0 µg DNA. The mixture was then incubated in a thermal cycler (PCR System 9700 Applied Biosystems) using the following amplification profile: denaturation at 95°C for 5 min, 40 cycles at 95°C for 40 s, 55°C–57°C for 40 seconds and 72°C for 2 min, and a final extension step for 10 min at 72°C. PCR products were verified by electrophoresis on 2% agarose gels.

For the single nucleotide primer extension assays, the SNP detection kit (SNaPshot™, Applied Biosystems) was used. This assay is based on a dideoxy nucleotide triphosphate (ddNTP) single base extension of an unlabelled oligonucleotide primer, which ends 5' to the SNP that will be typed. By using varying lengths of primers that detect each of the SNPs,

different SNPs can be typed in a multiplex reaction. In a first step, free primers and dNTPs of the PCR mixture were removed, by incubating 7.5 µl of the PCR reaction with 2.5 U shrimp alkaline phosphatase (SAP) (Amersham Biosciences) and 2 U of Exonuclease I (ExoI) (Amersham Biosciences) at 37°C for 1 hour. The enzymes were then inactivated at 75°C for 15 min. The extension reaction was prepared with 5 µl SNaPshot Multiplex Ready Reaction Mix, 2.3 µl of SAP/ExoI treated PCR products, and 0.2–1.25 µM of each unlabelled primer. The extension was performed in a thermal cycler (PCR System 9700, Applied Biosystems), using the following temperature profile: denaturation at 96°C for 10 s, annealing at 50°C for 5 s, and extension at 60°C for 30 s, for a total of 25 cycles. Unincorporated ddNTPs were then removed with 1 U of SAP for 1 hour at 37°C. The enzyme was finally inactivated at 75°C for 15 min. Then, 0.5 µl of the SNaPshot reaction was mixed with 9.25 µl of Hi Di™ formamide (Applied Biosystems) and 0.25 µl GeneScan™ 120 Liz™ size standard (Applied Biosystems). After denaturation at 95°C for 5 min, the mixture was resolved on an ABI PRISM® 3100 genetic analyser (Applied Biosystems) and analysed with GeneScan® analysis software (Applied Biosystems).

#### Statistical analysis

Database management and statistical analysis was performed with the SAS statistical package, release 8.01 (SAS Institute Inc. Cary, NC, USA), and the SYSTAT package, release 7.0 (SPSS Inc. Chicago, IL, USA). Statistical tests were considered significant when their type I error was less than 0.05. Differences among the means of FEV<sub>1</sub>% predicted values of Czech and Belgian populations were assessed using a two sample Student's *t* test after checking whether the variances of the populations were equal or not (using an *F* test) and adjusting the formula of the Student's *t* test for this. Comparison between one independent and one dependent qualitative variable were assessed using the  $\chi^2$  likelihood ratio test (for example, the distribution of patients having an FEV<sub>1</sub>% predicted above or below 70% as a dependent of the different genotypes), or by the Fisher exact probability test when more than 20% of the cells had an expected count of less than 5. When the independent variables were qualitative and the dependent variable was quantitative, a parametric analysis of variance (ANOVA) was done (for example, the

**Table 2** Single nucleotide extension primers for MBL2 SNPs

SNP*	Accession number	Nucleotide sequence (5'→3')†
Y/X (425 g/c)	AF080508	AAAAAAAAAAAAAAAAAAAAATGACGCGTCCCATTTGTCTCACTGCCAC
A/D (868 c/t)	AF080508	GCATCAACGGCTTCCAGGCAAGATGGG
A/B (875 g/a)	AF080508	CGGCTTCCAGGCAAGATGGGCGTGATG
A/C (884 g/a)	AF080508	AAAAAAAAAAAAAAAAACCTGGTCCCTTTTCT‡

\*The names of the alleles found at each polymorphic locus are given. The nucleotides for each allele are given in brackets; here the number refers to the polymorphic nucleotide position of the Genbank DNA sequence of the specified accession number given in the neighbouring column.

†The length of the oligonucleotide might be increased by the addition of an A-tail.

‡A reverse oligonucleotide is used.

distribution of mean FEV<sub>1</sub>% predicted values as a dependent of genotypes). The Pearson's correlation coefficient was calculated to test the goodness of fit of one independent quantitative value and one dependent quantitative value in a linear regression model (for example, the dependent value of FEV<sub>1</sub>% predicted against the independent value of age).

## RESULTS

The structural and promoter polymorphisms in the MBL2 gene (*A/O*, *Y/X* respectively) were typed in 179 cystic fibrosis patients and 187 controls. Hardy Weinberg equilibrium of the different SNPs was checked in the total Belgian patient, Belgian control, Czech patient, and Czech control groups. No Hardy-Weinberg disequilibrium was detected for any group of individuals. We tested for the possibility of population stratification because of differences in ethnicity, age, and sex. The allele or genotype frequencies of Belgian controls with respect to Belgian patients, of Czech controls with respect to Czech patients, of the Belgian control with respect to Czech control population, and of the Belgian patients with respect to Czech patients were not significantly different (table 3); the spirometric variables were distributed equally between Belgian and Czech patients with cystic fibrosis (respectively 68.3% and 68.8%;  $p = 0.92$ ); and the means of the spirometric values for each genotype did not differ significantly between the Belgian and Czech cystic fibrosis cohorts of age selected patients; therefore the data from the two populations were merged. FEV<sub>1</sub>% predicted values of patients with cystic fibrosis decline with age,<sup>13</sup> and girls have a steeper decline in pulmonary function than boys.<sup>14</sup> FEV<sub>1</sub>% predicted values were calculated according to Knudson, which takes into consideration age, sex, and height.<sup>12</sup> Nevertheless, we tested if the corrected FEV<sub>1</sub>% predicted values were truly independent of sex or age in the present age selected cohort of patients, by comparing the means of the FEV<sub>1</sub>% values between male and female patients, and calculating the Pearson's correlation coefficient for age versus FEV<sub>1</sub>% predicted values. No influence of age ( $r^2 = 0.005$ ) or sex ( $p = 0.14$ , mean difference = 6.4, 95% confidence interval = -14.8 to 2.0, two sample student's *t* test) on the FEV<sub>1</sub>% values was found in the present age selected cohort of patients with cystic fibrosis.

We then tested if the values of FEV<sub>1</sub>% predicted of the cystic fibrosis patients were dependent on MBL2 genotypes (table 4). Given the fact that almost no functional MBL protein is found in individuals homozygous for the *O* allele, and very low MBL protein levels are found in individuals heterozygous for the *O* allele, a dominant model for the *O* allele was tested. At the mean age of our cohort of studied patients with cystic fibrosis (13.4 years), the average FEV<sub>1</sub>% in cystic fibrosis patients is approximately 70%. The group of patients having an FEV<sub>1</sub>% less than 70% was therefore compared with that having an FEV<sub>1</sub>% higher than 70%. The distribution of MBL2 *A/O* variants was however not

significantly different in patients having an FEV<sub>1</sub>% predicted to be either higher or lower than 70%. The mean FEV<sub>1</sub>% predicted values did not differ significantly between the *AA* and combined *AO+OO* genotypes ( $p = 0.10$ ), but a trend was observed in the relative reduction of lung function. Patients having the *AO* or *OO* genotype tended to have worse FEV<sub>1</sub>% predicted values than patients having the *AA* genotype. In association studies, severely affected patients are compared with mild patients. The discrimination between these two groups of patients to be used in association studies is, however, not straightforward. Only SNPs with a large contribution in modulation are very likely to be detected in association studies when the most severely affected patients are compared with the milder ones. Most probably however, cystic fibrosis lung disease is affected by a large number of modulating genes, each of them expected to have a modest contribution. In the latter case an association might not be detected when only the most severe patients are studied, since each SNP might not be present in all severely affected patients (false negatives in the severest group of patients), while it might be still present in milder patients (false positives in the milder group of patients). Moreover, a modulating role of an SNP might only become penetrant at higher ages. This is even more relevant given the increasing lifespan of cystic fibrosis patients. Therefore, when studying paediatric patients, no effect or only a modest effect might be seen, resulting in cystic fibrosis disease that is not yet classified as moderate or severe. However, an association might still be detected if the mildly affected patients are compared with both the severe and moderately affected patients, rather than the severely affected patients alone. A cutoff of 90% FEV<sub>1</sub> predicted value was therefore also used to discriminate between very mildly affected patients and all other patients with cystic fibrosis. When 90% FEV<sub>1</sub> predicted value was used for discrimination in lung disease severity, a significantly higher proportion of patients having an FEV<sub>1</sub> predicted value higher than 90% were homozygous for the *AA* genotype compared with patients having an FEV<sub>1</sub> predicted value lower than 90% ( $p = 0.002$ ). Specifically, the three *AO* patients having an FEV<sub>1</sub> higher than 90% carried the *AB* MBL2 genotype. In the group of patients having an FEV<sub>1</sub> lower than 90%, 22 were typed as *AB*, 5 had the *AC* genotype, 14 patients had the *AD* genotype, and 1 patient had the *BC* MBL2 genotype.

A combined test of the MBL2 *A/O* and MBL2 *Y/X* polymorphisms was also performed. The MBL2 gene high producers *AA-YY* and *AA-YX* were tested against the MBL2 gene low producers found in this study (*AA-XX*, *AO-XX*, *AO-XY*, and *OO-YY*) (table 4). The distribution of the combined genotypes was not significantly different in patients having >70% FEV<sub>1</sub> predicted compared with patients having <70% FEV<sub>1</sub> predicted, but was significantly different when 90% FEV<sub>1</sub> predicted was used as a cutoff. A significantly higher proportion of patients having an FEV<sub>1</sub> predicted value higher

**Table 3** Allele and genotype frequencies of MBL2 gene variants in patients with cystic fibrosis and control subjects

	MBL2 <i>A/O</i>					MBL2 <i>Y/X</i>				
	<i>AA</i>	<i>AO</i>	<i>OO</i>	<i>A</i>	<i>O</i>	<i>YY</i>	<i>YX</i>	<i>XX</i>	<i>Y</i>	<i>X</i>
Czech patients n = 122	72 (59.0)	47 (38.5)	3 (2.5)	191 (78.3)	53 (21.7)	64 (52.9)	51 (42.1)	6 (5.0)	179 (74.0)	63 (26.0)
Belgium patients n = 57	32 (56.1)	24 (42.1)	1 (1.8)	88 (77.2)	26 (22.8)	36 (63.2)	16 (28.1)	5 (8.8)	88 (77.2)	26 (22.8)
Total patients n = 179	104 (58.1)	71 (39.7)	4 (2.2)	279 (77.9)	79 (22.1)	99 (57.6)	63 (36.6)	10 (5.8)	261 (75.9)	83 (24.1)
Czech controls n = 102	62 (63.9)	31 (32.0)	4 (4.1)	155 (79.9)	39 (20.1)	53 (52.0)	43 (42.2)	6 (5.9)	149 (73.0)	55 (27.0)
Belgium controls n = 85	45 (52.9)	32 (37.6)	8 (9.4)	122 (71.8)	48 (28.2)	54 (63.5)	28 (32.9)	3 (3.5)	136 (80.0)	34 (20.0)
Total controls n = 187	107 (58.8)	63 (34.6)	12 (6.6)	277 (76.1)	87 (23.9)	107 (57.2)	71 (38.0)	9 (4.8)	285 (76.2)	89 (23.8)

For each allele or genotype, the number of alleles or individuals is given, percentages are given in brackets. The distribution of the genotypes for the patients with cystic fibrosis is given irrespective of age. There may be a difference in number of controls typed for the structural variants and promoter variants due to technical problems.

**Table 4** Distribution of MBL2 genotypes according to FEV<sub>1</sub>% in patients aged 12 to 15 years

	Genotype		Mean difference	95% confidence interval	p value
Structural variants	AA	AO+OO			
Mean FEV <sub>1</sub> % predicted (standard deviation)	71.8 (22.5)	64.8 (22.1)	7.0	-1.5 to 15.6	0.10
No>70% FEV <sub>1</sub> (%) n=59	37 (62.7%)	22 (37.3%)			0.51
No<70% FEV <sub>1</sub> (%) n=53	30 (56.6%)	23 (43.4%)			
No>90% FEV <sub>1</sub> (%) n=23	20 (87.0%)	3 (13.0%)			0.002
No<90% FEV <sub>1</sub> (%) n=89	47 (52.8%)	42 (47.2%)			
Structural or promoter variants	High*	Low†			
Mean FEV <sub>1</sub> % predicted (standard deviation)	72.2 (22.9)	65.3 (22.7)	6.9	-1.4 to 15.3	0.10
No>70% FEV <sub>1</sub> (%) n=59	34 (57.6%)	25 (42.4%)			0.36
No<70% FEV <sub>1</sub> (%) n=53	26 (49.1%)	27 (50.9%)			
No>90% FEV <sub>1</sub> (%) n=23	18 (78.3%)	5 (21.7%)			0.006
No<90% FEV <sub>1</sub> (%) n=89	42 (47.2%)	47 (52.8%)			

\*High is AA-YY+AA-YX.

†Low is AA-XX, AO-XX, AO-XY and OO-YY.

than 90% had the combined MBL2 high producing genotypes AA-YY and AA-YX ( $p = 0.006$ ).

For 116 cystic fibrosis patients we had records of the age at the first onset of *P. aeruginosa* colonisation. MBL2 gene structural variants alone, or in combination with MBL2 gene promoter variants, did not significantly associate with the age at the first infection with *P. aeruginosa* ( $p = 0.88$  and  $0.6$ , respectively).

## DISCUSSION

The patients in this study were derived from two white populations within Europe. We decided to group the patients, to strengthen the statistical power of the study. To exclude variables that might affect the phenotype, the following precautions were taken. Only patients homozygous for the F508 deletion were included, to exclude an effect of the CFTR genotype on disease. Patients from only two centres were included, to exclude variability in disease status caused by different treatments and variability in measurement of clinical variables. Only a small age group of patients was studied here. Indeed, the effect of a mutation might become only penetrant by age. Moreover, at any age, a patient might have a mild form of the disease, but it might still become severe when the patient is older. It is indeed important to neutralise the age variable as much as possible, since FEV<sub>1</sub>% predicted values of patients with cystic fibrosis declines more rapidly with age than in the general population, and therefore may interfere with results when testing pulmonary function as a dependent of genotype distribution. The fact that FEV<sub>1</sub>% values did not specifically differ with the age of the patients within the present cohort supports the credibility of the chosen age group. We also checked whether we could compare the disease severity between subjects without the influence of sex, since girls are known to have significantly lower FEV<sub>1</sub>% values than boys. However, we found no significant difference in corrected FEV<sub>1</sub>% values between male and female patients within the present cohort of age selected patients. All genotype distributions of patients and controls were in Hardy-Weinberg equilibrium ruling out population stratification of the patient groups. The frequencies of the tested genotypes and FEV<sub>1</sub>% values were not significantly different between the two populations, allowing us to pool the populations.

We found an altered distribution of the MBL2 gene polymorphisms, depending on pulmonary function. However, when only a limit of 90% FEV<sub>1</sub> predicted value was used, an effect of the MBL2 gene phenotype was observed. Specifically, AA homozygotes were found to

associate with a better pulmonary phenotype than AO heterozygotes and OO homozygotes. The AO and OO MBL2 genotypes are known to result in low MBL protein levels. Also, when the Y/X locus was further taken into consideration, high producers AA-YY and AA-YX MBL2 genotypes were found to have better pulmonary function than low producers AA-XX, AO-XY, AO-XX, and OO-YY MBL2 genotypes. It cannot be excluded that the observed association might be caused by another polymorphism in the MBL2 gene, or even a linked gene. However, given the well known functional biological consequences of the studied polymorphisms, it is very likely that the studied polymorphisms are directly involved.

The fact that we found no altered distribution of MBL2 gene polymorphisms depending on pulmonary function when 70% FEV<sub>1</sub> predicted value was used as a cutoff for discriminating pulmonary function, and a significant difference was found when the milder 90% FEV<sub>1</sub> predicted value was used instead, confirms our working hypothesis that modulating polymorphisms might not be fully penetrant at a given age. Therefore, at the average age of 13.4 years, MBL2 gene modulation of cystic fibrosis pulmonary disease is observed in severely and moderately affected patients in the present study, rather than in the severely affected patients alone. In a previous study, MBL2 gene polymorphisms seemed to have a stronger effect on pulmonary function, perhaps as a result of the older patient group studied. The range of ages of the previous study was 7–41 years with a median age of 16.2 years<sup>4</sup> compared with the patients of the present study, of 12–15 years with a median age of 13.3 years. Also, the earlier study had typed four times more patients as being homozygous for the O allele, which may influence results since these patients had an overall lower FEV<sub>1</sub>% value than patients typed as AO.<sup>4</sup> Another small study, that paired 11 cystic fibrosis patients typed as MBL2 AO and OO with 11 age and sex matched cystic fibrosis patients with the MBL2 AA genotype found a weak association of MBL2 gene variants with FEV<sub>1</sub>% predicted. The power of this study is questionable because of the small group of patients studied.<sup>5</sup>

No significant association was found between the age at first infection with *P. aeruginosa* and MBL2 A/O genotypes or combined A/O and Y/X genotypes. *P. aeruginosa* infection is the most common pulmonary infection in cystic fibrosis patients.<sup>15</sup> Progression of lung disease unequivocally accelerates after *P. aeruginosa* acquisition,<sup>16</sup> therefore, the younger the age of first infection with *P. aeruginosa*, the more rapid the decline in pulmonary function. Albeit patients with a very mild pulmonary phenotype associate with MBL2 high producers of protein, MBL protein does not seem to protect



our patients from *P. aeruginosa*. In a previous study, the differences in lung function between AA MBL2 patients and patients who were either AO or OO was found only in patients who had chronic *P. aeruginosa* infection.<sup>4</sup> However, in agreement with the present study, they found that MBL protein offered no protection against *P. aeruginosa* with respect to the prevalence of chronic infection or the age at onset of infection.<sup>4</sup> MBL is not directly secreted in the lungs, but is secreted into the circulation by the liver. It may well be that the high production of MBL protein protects patients by other means than against *P. aeruginosa*, and in this way the general health of these patients, enabling them to combat the manifestations of cystic fibrosis. Indeed, the presence of cirrhosis in cystic fibrosis patients is significantly associated with AO and OO MBL2 genotypes.<sup>17</sup> Liver cirrhosis would contribute to the worsening health of cystic fibrosis patients, weakening their general health.

A modulating role of a genetic factor with disease, based on association studies, should only be treated as tentative until the finding of an association has been confirmed in other studies. This study now confirms an association between MBL2 gene variants and the cystic fibrosis pulmonary phenotype, and it is therefore very likely that MBL2 is indeed a modulating factor in cystic fibrosis disease.

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